## REMARKS

Claims 30-39 are pending. Claims 30-39 are rejected. Claims 30, 33, 34, 35, 36, and 37 were amended. No new matter has been added by virtue of this amendment and its entry is respectfully requested.

### *Claim Rejections Under 35 U.S.C. § 112*

Claims 33 -39 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner asserts:

> Claims 33-37 depend from claim 30 and recite the limitation "wherein the pluripotent brain stem cell" in the second line of the claims. There is insufficient antecedent basis for this limitation in the claim. Claim 30 recites multipotent progenitor or precursor brain stem cells.

In response, Applicants have amended claims 33-37 to correct the antecedent basis. No new matter has been added by virtue of this amendment and its entry is respectfully requested.

Claim 37 was further rejected as the isolated stem cells are introduced into an animal subject. In response, Applicants have amended claim 37 to recite that multipotent brain stem cells are cultured *ex-vivo* in medium comprising insulin, putrescine, progesterone, selenite, pituitary extract, transferrin, serum, growth factors, and contact-limiting factors; wherein said cultured cells are introduced into a tissue in an animal subject. Support for this amendment is found, for example, on page 14, lines 1-33 through to page 16 and page 18, lines 23-33 through to page 19, lines 1-24. No new matter has been added by virtue of this amendment and its entry is respectfully requested.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claims 30-39 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

In response, Applicants have amended claim 30 to recite "human and murine." The claim amendments are deemed to satisfy the requirements under 35 U.S.C. § 112 and as such, the rejection is moot. The amendments to the claims are not to be construed as surrender of any subject matter. Applicants reserve the right to pursue the subject matter in one or more continuation or divisional applications.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

***Claim Rejections Under 35 U.S.C. § 112, Scope of Enablement***

Claims 30-39 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement.

Although Applicants respectfully disagree with the Examiner's assertions, in order to compact and expedite prosecution, Applicants have amended independent claim 30 to recite: An isolated culture of multipotent, progenitor or precursor human and murine brain stem cells containing a sub-population of cells that are immunonegative for glial fibrillary protein, nestin and TuJ1, appear as phase-bright very dense bodies and exhibit areas of very small punctate staining interspersed with regions that lack staining when counter-stained with propidium iodide; and, wherein the culture comprises Type II and Type III clones, that positively display markers for glial fibrillary acidic protein, nestin and TuJ1. Applicants submit that the amended claims now overcome the Examiner's rejections. Dependent claims 31-39 depend on independent claim 30 and as such encompass all of the claim limitations of claim 30. These amendments were made solely for purposes of expediting prosecution and are not meant to be construed as

surrender of any subject matter. Applicants reserve the right to further prosecute the subject matter in one or more Divisional or Continuation applications.

Support for these amendments is found throughout the specification. For example, Applicants have described the stem cells and identified the actual markers that identify these stem cells, i.e. Type I, II and II cells. Applicants also provide detailed morphological and phenotypic descriptions. For example, page 5, lines 11-31 describe the phenotypic and morphological data:

> Figure 1 shows phase contrast and electron microscopic images of type I, II, and III clones. Figures 1A, 1C, and IE are phase contrast images of type I, II and III clones of cultured adult brain cells, respectively. Figures 1B, 1D, and IF show type I, II and III spheres counterstained with propidium iodide, respectively. Scale bars for Figures 1A-F are 40, 30, 20, 30, 20, and 30 microns, respectively;
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> Figure 2 depicts the types of spheres found in the culture paradigm of the invention, and the generation conditions for the appearance and evolution of sphere types from brain;
>
> Figure 3 shows the phase and electron microscopic images of type II (A and B) and type III (C and D) spheres. Scale bars for Figures 3A-D are 10, 5, 15, and 2 microns, respectively;
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> Figure 4 shows immunostaining of early and late type II and type III spheres. Scale bars for Figures 4A, G and J are 10 microns, Figures 4B and 4C are 15 microns, Figures 4E and 4F are 30 microns, Figure 4H is 20 microns, and Figure 4I is 100 microns;
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> Figure 5 shows the evolution and proliferation of type II (Figure 5A and SC), and type III (Figures 5B and SD) spheres. Scale bars for Figures 5A and SB are 25 microns, and for Figures 5C and 5D are 10 microns;
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> Figure 6 shows type II and type III spheres from ROSA-26 transgenic mice. Scale bars for Figure 6A is 50 microns, and Figure 6B is 30 microns; and
>
> Figure 7 shows phase and electron microscopy of a type II adult mouse and type II adult human sphere. The adult mouse sphere is approximately 100 microns in diameter, while the adult human sphere is approximately 200 microns in diameter.

Applicants describe the isolation of these cells, see, for example page 6, lines 5-23:

> Cell separation and cell adhesion can be manipulated using a variety of contact-limiting and contact-inhibiting factors. For example, chemical-separating agents such as mercaptoethanol, physical separating agents such as methylcellulose, and anti-adhesives such as poly 2-hydroxyethyl methacrylate are used to deter cell-cell and cell-substrate associates during the initial isolation of stem/precursor cells from the newly-dissociated brain. This allows the "purification" of these cells from mature, differentiated neurons and glia that are also dissociated during the brain dissociation procedures. The mature, differentiated neurons and glia cannot survive these anti-adhesion, anti-cell interaction procedures. Thus, agents such as mercaptoethanol are always used in the first stage of isolation of type I and II clones to help deter the survival of the more mature cellular elements (by deterring their clustering). At the same time, agents such as mercaptoethanol may have certain growth-promoting actions on the single stem/precursor cells that eventually proliferate to form these early sphere types.
>
> Since cell-cell and cell-substrate interactions are important for cellular differentiation, contact-inhibiting (or contact-limiting) factors as mercaptoethanol are eventually removed from the culture medium for the evolution or differentiation of type II and type III spheres.
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> The differentiation of type m spheres requires other additional factors, including growth factors like beta fibroblast growth factor, epidermal growth factor, or such factors that are also contained within pituitary extract. Such additional factors are described in the type III culture media discussed below (see, Example 3).

Further Applicants describe the types of purified stem cells. See, for example, page 7, lines 16-33 through to page 12, lines 1-6. See, also Examples 1-3 beginning on page 14 through to page 16, lines 1-5.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

## CONCLUSION

Applicants have made every effort to present claims which overcome the Examiner's assertions, and it is believed that all claims are now in condition for allowance. However,

Applicants request that the Examiner call the undersigned (direct line 561-671-3666) if anything further is required by the Examiner prior to issuance of a Notice of Allowance for all claims.

Applicants respectfully request entry of the foregoing amendments and remarks and reconsideration and withdrawal of all rejections. It is respectfully submitted that this application with claims 30-39 is in condition for allowance. If there are any remaining issues or the Examiner believes that a telephone conversation with the Applicants' attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at telephone number shown below.

Although, Applicants believe that no further extensions of time are required with submission of this paper, Applicants request that this submission also be considered as a petition for any extension of time if necessary. The Commissioner for Patents and Trademarks is hereby authorized to charge the amount due for any retroactive extensions of time and any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing or during prosecution of this application to Deposit Account No. 50-0951.

Respectfully submitted,

AKERMAN SENTERFITT

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Nicholas A. Zachariades
Registration. No. 56,712
P.O. Box 3188
West Palm Beach, FL 33402-3188
Tel: 561-653-5000

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